Overwinter Survival of *Colletotrichum acutatum* in Infected Strawberry Fruit in Ohio

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ABSTRACT

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Colletotrichum acutatum, a causal organism for anthracnose fruit rot of strawberry, was recovered from 100% of all infected strawberry fruit exposed for 18 wk in the laboratory to constant temperatures of −12 and −30 C or six cycles of fluctuating temperatures of −12 or −30 C for 2 wk followed by 5 C for 1 wk. In a 2-yr field study (1988-89, 1989-90), *C. acutatum* was recovered from nearly 100% of fruit located on the soil surface or 5–8 cm below the soil surface after exposure to winter conditions for 3 mo (November through January), but recovery of *C. acutatum* decreased with time after 3 mo. After 6 mo of exposure, from November through May over a 3-yr period, the percentage recovery of *C. acutatum* from nonmummified fruit located on and below the soil surface, respectively, was: 1988-89, 80 and 67%; 1989-90, 60 and 0%; and 1990-91, 7 and 7%. Percentage recovery of *C. acutatum* was: 1989-90, 53 and 7%, and 1990-91, 47 and 40% from mummified fruit located above and below ground, respectively. This is the first report of *C. acutatum* overwintering in a northern strawberry production region.

Anthracnose fruit rot of strawberry (*Fragaria* × *ananassa* Duchesne), caused by *Colletotrichum acutatum* J. H. Simmonds, was first observed in Australia (8). In the United States, *C. acutatum* was first reported from Mississippi in 1986 (9) followed by subsequent reports from Florida, Missouri, and California. The disease is now endemic throughout the major strawberry production areas in the southeastern United States and California and represents a major limitation to commercial fruit and nursery plant production (10).

Anthracnose fruit rot, caused by *C. acutatum*, was first observed in Ohio in 1985 but was first attributed to *C. gloeosporioides* (Penz.) Penz. & Sacc. in Penz (3). *C. acutatum* was subsequently identified on rotten fruit in Holmes County, Ohio, in 1990, and at several locations across the state in 1991 (M. A. Ellis, *unpublished*). Although the disease has generally been considered to be restricted to the warmer regions of the southern United States and California, reports of anthracnose fruit rot are becoming increasingly common in the north-central and northeastern United States. We consider the disease to be a major threat to strawberry production in the northern production areas of the United States.

The most likely means of pathogen introduction into uninfested production fields is on infected or infested nursery plants. Disease spread occurs through rain splash dispersal of conidia (13). Once the pathogen is established in a field, its ability to survive the winter under northern (Ohio) conditions is of major concern. In addition, the increased establishment of day-neutral or everbearing strawberry plantings in Ohio greatly reduces the survival period required for *C. acutatum* between fruit crops. Day-neutral varieties produce fruit from spring up to the first killing frost. In perennial plantings of day-neutral varieties, fruit could be present in the planting from May through October or early November. Eastburn and Gubler (2) reported that *C. acutatum* survived in buried strawberry tissue for 9 mo under California conditions, but soil population densities gradually decreased over an 11-mo period. The overwinter survival of *C. acutatum* in strawberry fields under colder winter conditions in northern production areas has not been previously studied.

The purpose of this study was to determine the ability of *C. acutatum* to survive the winter on infected strawberry fruit placed on and below the soil surface under Ohio conditions.

MATERIALS AND METHODS

Preparation of infected fruit. The isolate of *C. acutatum* used throughout the study was obtained from an infected strawberry fruit collected near Mt. Vernon, Ohio, in 1985. To maintain pathogenicity, strawberry fruit were inoculated and the fungus reisolated from infected fruit every 2 wk.

Immature (white stage) (1) strawberry fruit (variety Midway) with pedicels intact were collected from plants grown in the greenhouse. Fruit were washed with distilled water, surface-disinfested by soaking in 70% EtOH for 60 sec, and rinsed in distilled water. Fruit were then placed on elevated screens (6-mm mesh) in 5-L plastic containers (12) that were filled with 700 ml of deionized water to a level just below the screen. The pedicel

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of each fruit was extended through the screen so that the pedicel tip was immersed in water in order to reduce desiccation of inoculated fruit. Fruit were inoculated by spraying them to runoff with a conidial suspension of *C. acutatum* (5×10^4 conidia per milliliter) prepared by scraping and washing conidia from the surface of potato-dextrose agar (PDA) culture dishes after 4 days of incubation at 25 C in the dark (12). Conidia were suspended in deionized water and the concentration adjusted using a hemacytometer (11). Containers were closed and incubated at 25 C for 24 hr, after which they were opened and placed back in the incubator for an additional 6–8 days. Infected fruit with typical anthracnose symptoms were selected for use. Mummified fruit were prepared by placing infected fruit in dry containers on the laboratory bench and allowing them to air-dry for 3 wk.

Effect of freezing on pathogen survival. Mummified and nonmummified infected fruit were placed separately into nylon mesh bags (five fruit per bag) and the bags were closed with staples (2). Bags containing each fruit type were stored at 5 C for 3 days, then samples were placed in separate freezers maintained at −12 and −30 C. Three bags (subsamples) of each fruit type per temperature were stored at constant temperature for 18 wk. To observe the effects of alternate freezing and thawing, three additional bags of each fruit type were maintained at each freezing temperature (−12 and −30 C) for 2 wk and then at 5 C for 1 wk before returning them to their respective freezing temperatures. This cycle was repeated six times (18 wk). Then, all bags were placed at 5 C for 3 days. Sections of each fruit from all bags were placed onto modified dextrose peptone yeast extract agar (DPYA) (2,7) and PDA in culture dishes. Dishes were incubated at 25 C for 4–8 days in the dark, and the recovery of *C. acutatum* was recorded based on colony morphology as well as conidia shape and size (8,10).

Overwinter survival of *C. acutatum* in infected fruit. In November 1988, infected (nonmummified) strawberry fruit (variety Midway) were prepared and placed in nylon mesh bags as previously described. Mummified fruit were not used during the first year of the study. Nylon bags (replicates) containing five fruit each were placed on the soil surface or buried 5–8 cm below the surface. All samples were covered with 8 cm of straw mulch on the soil surface in order to more closely duplicate Ohio field conditions (6). Three bags were placed at each position (on the ground or buried) for each of six sampling dates. Samples were taken at 1-mo intervals from 15 December 1988 through 15 May 1989. Infected fruit were removed from the bag, washed in running water for 30 min (2), surface-disinfested by soaking in 70% EtOH for 60 sec, and finally rinsed in sterile deionized water. Sections of tissue from each fruit were placed on DPYA and PDA containing 30 mg/L of streptomycin sulfate (2,7). Dishes were incubated as previously described and the recovery of *C. acutatum* recorded based on conidia morphology and colony morphology.

The experiment was repeated in 1989 as previously described with the following exceptions. In addition to infected fruit, mummified fruit were prepared as previously described and used throughout the experiment. Thus, for each sample date and position, three bags of infected nonmummified fruit and three bags of infected mummified fruit were used. Fruit samples were placed in the field in November 1989, and samples were collected at 1-mo intervals from 15 January 1990 through 15 May 1990. Upon recovery from the field, mummified fruits were soaked in 6 ml of sterile distilled water for 1 hr with occasional agitation. After 1 hr, a 0.5-ml aliquot of the soak water was spread on the surface of DPYA and PDA containing 30 mg/L of streptomycin sulfate in petri dishes. Dishes were incubated and the recovery of *C. acutatum* recorded as described above.

In 1990, infected mummified and nonmummified fruits were prepared as previously described and placed in the field during November. All fruit were collected and assayed for the presence of *C. acutatum* on 15 May 1991. Weather data for all 3 yr were obtained from a standard meteorological station located at the Ohio Agricultural Research and Development Center in close proximity (0.25 km) to the test plot (Table 1).

RESULTS AND DISCUSSION

The effects of constant or fluctuating freezing temperatures alone had no apparent effect on recovery of *C. acutatum* from mummified or nonmummified fruit after 18 wk of exposure in laboratory tests. *C. acutatum* was recovered from all fruit used in this study. The temperatures of −12 and −30 C and exposure time of 18 wk were selected because they represent extreme temperature conditions that far exceed those to which naturally infected fruit would be exposed to under winter conditions in Ohio. Results of this study indicate that cold winter temperatures alone are not effective in killing *C. acutatum* in infected strawberry fruit tissues.

Results from field studies also demonstrated that the temperatures during three winters in Ohio (Table 1) were not sufficient to kill the fungus in tissues of infected fruit left in the field. Although average monthly air temperatures were only occasionally below 0 C, many individual days had subfreezing temperatures. After 3 mo of exposure in 1988-89 and 1989-90, *C. acutatum* was recovered from nearly 100% of all fruit regardless of location (Table 2). In 1988-89, *C. acutatum* was recovered from 67 and 80% of all buried and surface (nonmummified) fruit, respectively, after 6 mo of exposure (the entire winter). This suggests that once anthracnose fruit rot is established, infected fruit that are left in the field provide an excellent source for overwintering inoculum.

We included mummified fruit in the 1989-90 field study in response to our observation that strawberry fruit infected with *C. acutatum* often dry down to form shriveled mummies under natural field conditions. Fruit mummies are implicated in overwinter survival of several plant pathogens and have been demonstrated to be an important source of overwintering inoculum for *Phytophthora cactorum* (Leb. & Cohn) Schroet., the causal agent of strawberry leather rot, in Ohio strawberry fields (4). Mummies that developed from fruit infected by *P. cactorum* in late June or early July have been observed to survive intact on the soil surface until the following season (4).

In 1988-89, a decrease in percentage recovery was observed in most treatments after 6 mo of exposure, especially

Table 1. Mean daily ambient air temperature, soil temperature, and total monthly precipitation for 30-day sampling periods from November through May of 1988-89, 1989-90, and 1990-91, Wooster, Ohio

Sampling period (month/day)	Ambient air temperature (C)				Soil temperature (C)				Total precipitation (mm)			
	1988-89	1989-90	1990-91	LTA[a]	1988-89	1989-90	1990-91	LTA	1988-89	1989-90	1990-91	LTA
11/15 to 12/15	1.4	2.9	4.9	1.8	4.0	4.6	6.1	4.6	31.5	45.2	47.5	74.7
12/15 to 1/15	−6.3	−0.7	−0.1	−2.5	0.5	1.8	3.2	1.4	29.2	52.8	140.0	73.9
1/15 to 2/15	2.4	1.1	−1.0	−3.2	2.7	2.2	1.9	1.0	86.9	27.2	41.3	73.9
2/15 to 3/15	0.6	−2.8	0.8	0.1	2.7	0.7	2.4	2.5	57.9	47.6	42.2	70.9
3/15 to 4/15	6.8	5.6	8.9	5.7	7.5	6.2	8.0	6.5	53.1	65.5	68.1	89.2
4/15 to 5/15	12.4	8.9	14.2	11.8	12.8	10.2	14.5	13.1	59.4	50.3	46.0	86.9

[a] Long-term average, air temperature (91 yr), soil temperature (10 yr), and rainfall (77 yr).

Table 2. Recovery of *Colletotrichum acutatum* from infected mummified and nonmummified strawberry fruit located below (5–8 cm) or on the soil surface following exposure to winter conditions in Ohio[a]

		Nonmummified fruit				Mummified fruit			
		Buried		Aboveground		Buried		Aboveground	
Sampling date (month/year)	Exposure time (mo)	Recovered fruit (no.)	Percentage recovery of *C. acutatum*	Recovered fruit (no.)	Percentage recovery of *C. acutatum*	Recovered fruit (no.)	Percentage recovery of *C. acutatum*	Recovered fruit (no.)	Percentage recovery of *C. acutatum*
1988–89									
12/88	1	15	100 (0)[b]	15	100 (0)	...[c]	...	...	...
1/89	2	15	100 (0)	15	100 (0)	...	...	...	...
2/89	3	15	93 (6)	15	100 (0)	...	...	...	...
3/89	4	15	67 (12)	15	80 (10)	...	...	...	...
4/89	5	15	67 (12)	15	80 (10)	...	...	...	...
5/89	6	15	67 (12)	15	80 (10)	...	...	...	...
1989–90									
1/90	2	15	100 (0)	15	100 (0)	15	100 (0)	15	100 (0)
2/90	3	15	100 (0)	15	100 (0)	15	100 (0)	15	100 (0)
3/90	4	15	60 (13)	15	87 (9)	13	87 (9)	15	100 (0)
4/90	5	12	33 (12)	11	60 (13)	9	53 (13)	8	47 (13)
5/90	6	0	0 (0)	13	60 (13)	1	7 (6)	8	53 (13)
1991									
5/91	6	4	7 (6)	1	7 (6)	6	40 (13)	7	47 (13)

[a] All figures are based on three replications of five fruit (each) for each fruit type, position, and sampling date.
[b] Percentage of 15 fruit with *C. acutatum* and standard error in parentheses.
[c] No data; mummified fruit were not used in 1988–89.

for buried fruit (Table 2). This was partly due to decomposition of fruit over time to the point that intact fruit could not be retrieved. *C. acutatum* was recovered from 0 and 7% of the buried nonmummified and mummified fruit, respectively. However, recovery rates from fruit on the ground were 60 and 53% for nonmummified and mummified fruit, respectively.

In 1990-91, *C. acutatum* was recovered from 7% of the buried and aboveground nonmummified fruit after 6 mo of exposure. The fungus was recovered from 40 and 47% of buried and surface mummified fruit for the same exposure period. As with the previous year, decomposition of fruit was partly responsible for the reduction in fungal recovery.

Our studies did not determine which propagules of the fungus survived in infected strawberry fruit. Appressoria, chlamydospore-like hyphal cells, and conidia have all been reported to serve as survival propagules for *C. acutatum* (2,5).

These results clearly demonstrate the ability of *C. acutatum* to survive the winter in strawberry fields under Ohio conditions. Our data indicate that survival is greatest in infected fruit located on the soil surface. Because of the lack of cultivation in perennial production schemes in the northern production areas, most infected fruit left in the field would remain above the soil surface. In certain situations in Ohio, growers have plowed down fields in attempts to eradicate the pathogen. Our data suggest that deep plowing may be beneficial in some years in reducing pathogen populations in the field.

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